Human Metapneumovirus: A New Player among Respiratory Viruses

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The human metapneumovirus (hMPV) is a newly described member of the *Paramyxoviridae* family belonging to the *Metapneumovirus* genus. Since its initial description in 2001, hMPV has been reported in most parts of the world and isolated from the respiratory tract of subjects from all age groups. Despite the fact that prospective and case-control studies have been limited, the epidemiology and clinical manifestations associated with hMPV have been found to be reminiscent of those of the human respiratory syncytial virus, with most severe respiratory tract infections occurring in infants, elderly subjects, and immunocompromised hosts. Additional research is needed to define the pathogenesis of this viral infection and the host's specific immune response.

Acute respiratory tract infections (ARTIs) are a major cause of morbidity and mortality worldwide. A variety of viruses, including the influenza viruses, human respiratory syncytial virus (hRSV), parainfluenza viruses, adenoviruses, rhinoviruses, and coronaviruses, have been associated with clinical syndromes ranging from mild illnesses, such as the common cold, to more severe devastating conditions, such as pneumonia. However, ~50% of the cases of community-acquired pneumonia in adults and 15%–35% of cases of bronchiolitis and pneumonia in pediatric patients are still without a known etiology [1–3]. Along with inadequate diagnostic methods, this observation also suggests that other respiratory tract infectious agents may exist and remain to be identified. A new respiratory virus, the human metapneumovirus (hMPV) was first isolated in 2001 from nasopharyngeal aspirates (NPAs) obtained from young children in The Netherlands [4]. On the basis of morphological, biochemical, and genetic features, hMPV was tentatively classified with avian metapneumovirus (APV), the causative agent of an upper respiratory tract disease in turkeys and other birds [5], in the *Metapneumovirus* genus. The latter, together with the *Pneumovirus* genus (containing hRSV as the type species), is part of the *Pneumovirinae* subfamily within the *Paramyxoviridae* family.

Received 23 October 2003; accepted 17 December 2003; electronically published 15 March 2004.

Financial support: Canadian Institutes of Health Research (grant MOP-62789 to G.B.).

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Clinical Infectious Diseases 2004; 38:983–90

1058-4838/2004/3807-0011$15.00

VIROLOGICAL CHARACTERISTICS

Initial electron microscopic examination of hMPV isolates revealed morphological characteristics consistent with paramyxoviruses. Pleomorphic, spherical, and filamentous particles could be observed (figure 1) [6]. Spherical enveloped particles vary in size, with a mean diameter of 209 nm. The nucleocapsid has a length varying from <200 to ~1000 nm and a diameter of 17 nm.

HMPV isolates were initially found to grow on tertiary monkey kidney and LLC-MK2 (Rhesus monkey kidney) cells, with poor or no viral growth on other cell lines, including Madin Darby canine kidney, Vero (African green monkey kidney), human laryngeal carcinoma (HEp-2), human foreskin fibroblast, human rhabdomyosarcoma, transformed human kidney (293), human lung adenocarcinoma (A-549), and human colon adenocarcinoma (HT-29) [4, 7]. In addition, hMPV isolates did not exhibit hemagglutinating activity when tested with human, turkey, chicken, or guinea pig RBCs, which is consistent with other members of the Pneumovirinae subfamily [4, 7].

Intranasal inoculation of ferrets and guinea pigs with hMPV isolates did not cause any clinical symptoms [4]. Also, experimentally infected birds (juvenile turkeys and chickens) did not show clinical signs or virus replication over a 3-week period. On the other hand, there was evidence of efficient hMPV rep-

lication in the respiratory tract of experimentally infected monkeys (cynomolgus macaques) associated with mild upper respiratory tract signs [4]. Thus, in contrast to APV, hMPV is a respiratory pathogen of primates.

GENOMIC ORGANIZATION

The hMPV genome consists of a single negative strand of RNA of ~13 Kb containing genes coding presumably for a nucleoprotein (N), phosphoprotein (P), matrix (M) protein, fusion (F) protein, transcription elongation factor/RNA synthesis regulatory factor (M2), small hydrophobic (SH) surface protein, major attachment (G) glycoprotein, and major polymerase (L) subunit, arranged in the order 3′-N-P-M-F-M2-SH-G-L-5′, similar to APV [8, 9]. Two major differences exist between hMPV and hRSV genomes: the gene order is different, and hMPV does not contain nonstructural genes (figure 2) [10]. The possible relevance of the absence of these viral proteins, which have been associated with IFN antagonism by hRSV [11], deserves additional study. For hRSV, the G and F surface proteins are known as the major protective antigens [11], and this also needs to be verified for hMPV.

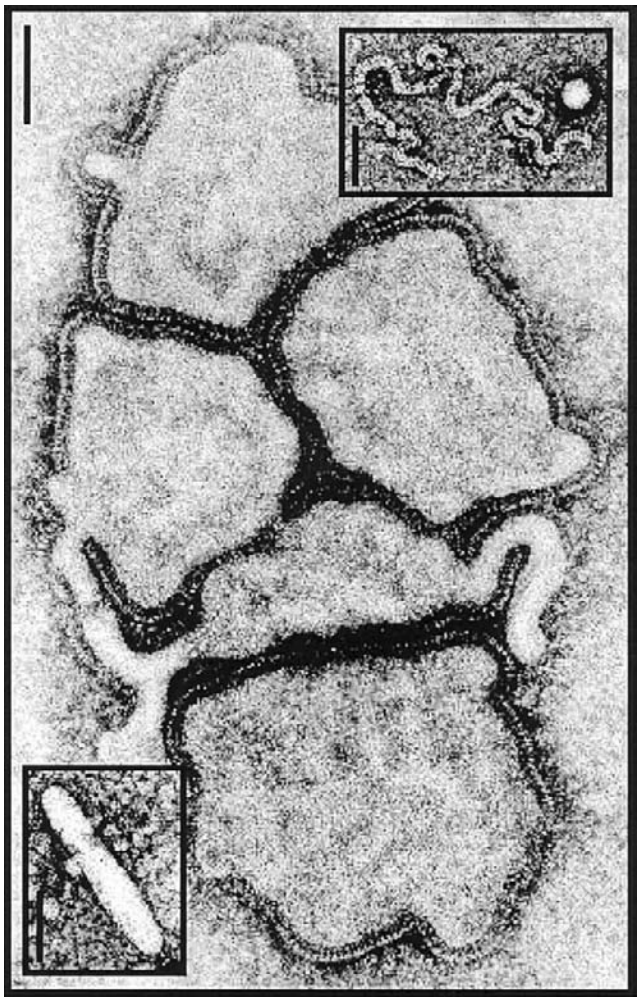

Figure 1. Negative-stain electron micrographs of human metapneumovirus (hMPV). Center image shows 5 pleomorphic hMPV particles; note the projections along the periphery of the viruses. Upper right and lower left insets show the nucleocapsid and the filamentous rodlike particle, respectively. Staining was done with 2% phosphotungstic acid. Bar markers represent 100 nm. From [6]; reproduced with permission from The University of Chicago Press.

Phylogenetic analyses have demonstrated that, among members of the *Pneumovirinae* subfamily, hMPV was most closely related to APV serotype C, the avian metapneumovirus that emerged in the United States in the late 1990s. For instance, the amino acid sequence identity between hMPV strains and APV-C and hRSV representatives varies from 66% to 89% and from 23% to 43%, respectively, when comparing the N, P, M, and F genes [4, 12]. The SH and G amino acid sequences of hMPV show important variability and share only 28% and 21% identity with their respective APV-C counterparts [8, 13, 14]. It is possible that this important divergence may account for the specific host range of these 2 related metapneumoviruses. Genetic analysis on a large number of hMPV isolates has identified 2 major groups and 2 minor genetic clusters within each group [6, 7]. However, additional investigations are still required to determine whether these genotypes represent different antigenic groups. Recently, the complete nucleotide sequences of hMPV isolates belonging to these 2 major groups were determined [9]. Nucleotide and amino acid sequence identities between the 2 hMPV groups were found to be 80% and 90%, respectively, which is similar to what has been reported with hRSV A and B genotypes.

EPIDEMIOLOGY

Since its initial report by the Dutch researchers in 2001, hMPV has been found in most parts of the world, with reports from North America (United States and Canada), Europe (United Kingdom, France, Germany, Italy, Spain, and Finland), Asia (Hong Kong and Japan), and Australia (table 1). The virus has also been identified in HIV-infected and nonimmunocompromised children from South Africa [15]. The few seroprevalence surveys from The Netherlands [4], Japan [16], and Israel [17] have indicated that virtually all children are infected by 5–10 years of age. In addition, studies have shown that hMPV is not a new pathogen, with serological evidence of human infection dating from 1958 in The Netherlands [4] and viral isolation for the past 10–20 years in Europe and Canada [4, 7]. Cases of severe hMPV infection in adults [7] and of reinfection in immunocompromised subjects [18] suggest that, despite universal infection in childhood, new infections can occur throughout life due to incompletely protective immune responses and/or acquisition of new genotypes. Surveys have in-

dicated that hMPV has a seasonal distribution overlapping hRSV circulation, with most cases reported during the winter/early spring months (table 1). Although most studies have limited their surveillance to the typical respiratory virus period, our group has shown that 87% of hMPV isolates recovered in cell culture in Quebec, Canada, were recovered during the period of December through May [7]. In addition, we found that outbreaks of hMPV infection tend to peak in early spring over a 4–6 week period, slightly later than outbreaks of hRSV infection, which also are more spread out in time [19]. However, additional studies over multiple years are needed to better define the seasonality of hMPV infection.

The role of hMPV as the cause of ARTI has been evaluated in many studies, mostly using molecular detection methods (table 1). Young hospitalized children have been best studied, and hMPV has been generally found in 5%–10% of ARTI cases in that population. However, in one study from Italy, the number of hMPV infections varied widely over a 3-year period and were associated with 7%–43% of hospitalizations for ARTI [20]. The relative role of hMPV in respiratory syndromes of adults has been less studied. In one study from Rochester, New York, hMPV was detected by serological tests and/or RT-PCR in 4.5% of young and elderly adults with ARTI [21]. Interestingly, evidence of hMPV infection was also found by serological testing in almost the same percentage (4.1%) in asymptomatic adults, raising the question of the causative role of hMPV in ARTI in adults. Recent studies by our group indicate that hMPV is rarely identified by RT-PCR in NPAs obtained from asymptomatic young children, with a rate significantly lower than that of subjects with ARTI (<1% vs. 6%) [19, 22]. It is interesting to note that the rates of detection of hMPV have been generally higher in retrospective than prospective studies (table 1), an observation consistent with some selection bias. Thus, to better define the role of hMPV in various respiratory conditions, large prospective studies using appropriate controls need to be conducted. In addition, testing of all clinical samples (i.e., not only those samples that are found to be negative for other viruses) must be performed. Nevertheless, most studies have so far indicated that hMPV is a frequent cause of severe ARTI, especially in young children.

CLINICAL MANIFESTATIONS

Table 2 summarizes the clinical findings of the first 28 hMPV-infected Canadian subjects retrospectively identified through our virology laboratory. Symptoms of both upper and lower respiratory tract infections have been associated with hMPV in young children, although most reports are biased towards description of the most severe symptomatology in hospitalized subjects. In young hospitalized children, the clinical features associated with hMPV infections are very similar to those of hRSV [19, 23–25]. Diagnoses of bronchiolitis, with or without pneumonitis, have been most commonly reported. A substantial proportion (up to 50%) of infected children also has concomitant otitis media [19]. Acute wheezing and asthma exacerbation have been associated with hMPV in some [23, 26] but not all [27] studies. Compared with hRSV infections, hMPV cases tend to occur at an older age [19, 26, 28]. The clinical outcome after hRSV infection has been more severe than that after hMPV infection when looking at the proportion of patients with hypoxemia, those with pneumonia, and those admitted to the intensive care unit in studies from Canada [19] and Europe [25, 28]. In contrast, researchers from Hong Kong found that hMPV infection was associated with a longer hospital stay and more cases of pneumonia than was hRSV infection [26]. The latter group reported an estimate of 442 hMPV-associated hospitalizations per 100,000 children aged ≤6 years. Children with underlying medical conditions may have more-severe hMPV disease, leading to hospitalization. In studies from North America, 25%–33% of hMPV cases occurred in children with underlying conditions, such as prematurity, cardiopulmonary problems, and immunosuppression [7, 29].

hMPV has been associated with flulike illnesses and colds in healthy adults (table 2) [7]. Stockton et al. [30] identified hMPV in 9 (2.2%) of 408 nasal and throat swabs obtained from subjects with flulike illnesses (i.e., patients who tested negative for influenza and hRSV RNA) seen by general practitioners in England. Eight of the 9 infected patients in that study were adults, and 6 of these patients also had clinical evidence of lower respiratory tract involvement. Falsey et al. [21] found a higher rate of hMPV illness among young adults, although older adults

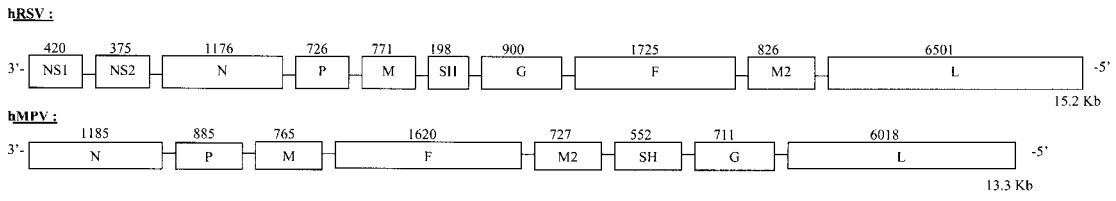

Figure 2. Schematic representation of the genomic organization of members of the *Pneumovirus* genus (represented by the human respiratory syncytial virus [hRSV]; GenBank accession no. NC 001781) and the *Metapneumovirus* genus (represented by the human metapneumovirus [hMPV]; GenBank accession no. AF371337). The lengths of the genomes are ~15 Kb and ~13 Kb for hRSV and hMPV, respectively. Length of each gene (in nucleotides) is also indicated. The overlap between the M2 and L genes of hRSV is not indicated.

Table 1. Incidence of human metapneumovirus (hMPV) infection in several studies.

Reference	Country	Year of report (period)	Type of study	Population	hMPV detection, *n/N* (%)[a]	Comments
[4]	The Netherlands	2000 (winter)	Retrospective/selected[b]	Children with ARTI	7/68 (10)	…
[42]	Australia	2001 (year-long)	Retrospective/selected[b]	Children with ARTI	3/200 (1.5)	…
[7]	Canada	2001 (winter)	Laboratory-based	Persons of all ages	20/862 (2.3)	Detection by culture
[30]	United Kingdom	2001 (winter)	Prospective/selected[b]	Persons of all ages with ILI	9/405 (2.2)	Community surveillance
[23]	Finland	2000–2001 (Sep–Jun)	Prospective	Hospitalized children aged <16 years with wheezing	10/132 (9)	3 of 10 patients infected with hMPV had coinfection
[24]	France	2001–2002 (Nov–Mar)	Retrospective/selected[b]	Hospitalized children with ARTI	19/337 (6.6)	…
[29]	United States	2001–2002 (Nov–Mar)	Laboratory-based/selected[b]	Children aged <5 years	19/296 (6.4)	2 of 19 patients infected with hMPV had coinfection
[43]	Spain	2000–2002 (Nov–Feb)	Prospective/selected[b]	Children aged <3 years with ARTI	6/147 (4.1)	…
[19]	Canada	2001–2002 (Dec–May)	Prospective/case-control	Hospitalized children aged ⩽3 years with ARTI	12/208 (5.8)	2 of 12 patients infected with hMPV had coinfection; 0 of 51 control subjects tested positive for hMPV
[27]	Australia	2001–2002 (summer and winter)	Prospective	Children aged <16 years with asthma and URTI	3/179 (2)[c]; 9/150 (6)[d]	…
[26]	Hong Kong	2001–2002 (year-long)	Prospective	Hospitalized children aged ⩽18 years with ARTI	32/587 (5.5)	2 of 32 patients infected with hMPV had coinfection
[21]	United States	1999–2001 (winter)	Prospective/case-control	Cohorts of young and elderly subjects with ARTI	44/984 (4.5)	Detection by seroconversion or PCR; 9 (4.1%) of 217 control subjects had seroconversion
[20]	Italy	2000–2002 (mostly winter)	Retrospective	Hospitalized children aged ⩽2 years with ARTI	23/90 (25)	Rate of positivity varied from 7% to 43%, according to year of study; 9 (39%) of 23 patients infected with hMPV had coinfection
[25]	Germany	2002 (Jan–May)	Retrospective	Hospitalized children aged ⩽2 years with ARTI	11/63 (17.5)	Detection by nested PCR; 3 of 11 patients infected with hMPV had hRSV infection
[31]	Canada	2001–2002 (winter)	Laboratory-based/selected[b]	Persons of all ages with ARTI	66/445 (14.8)	…
[28]	The Netherlands	2000–2002 (year-long)	Laboratory-based	Persons of all ages with ARTI	48/685 (7.0)	6 of 48 patients infected with hMPV had coinfection

NOTE. ARTI, acute respiratory tract infection; hRSV, human respiratory syncytial virus; ILI, influenza-like illness; URTI, upper respiratory tract infection.

[a] Data are no. of patients in whom hMPV was detected/no. of patients tested (%). hMPV detection was performed by RT-PCR unless specified otherwise.

[b] Only samples that were found to be negative for other respiratory viruses were tested for hMPV.

[c] Children with asthma.

[d] Children with URTI.

Table 2. Clinical findings in 28 human metapneumovirus (hMPV)–infected Canadian subjects, by age group.

Variable	Age group		
	0–4 years (n = 12)	15–65 years (n = 6)	>65 years (n = 10)
Underlying illness, no. (%) of subjects			
Immunosuppressive condition	2 (16.7)	2 (33.3)	2 (20.0)
Chronic respiratory disease	0 (0)	0 (0)	3 (30.0)
Chronic cardiovascular condition	0 (0)	1 (16.7)	4 (40.0)
Other	2 (16.7)	1 (16.7)	3 (30.0)
Hospitalization related to hMPV infection			
No. (%) of subjects hospitalized for respiratory conditions	11 (91.7)	2 (33.3)	6 (60.0)
Duration of hospitalization, median days (range)	4 (2–13)	35.5 (29–42)	10.5 (6–23)
No. (%) of subjects in nursing homes or hospitalized for unrelated conditions	1 (8.3)	2 (33.3)	4 (40.0)
ICU stay			
No. (%) of subjects hospitalized in ICU	3 (25.0)	1 (16.7)	2 (20.0)
Duration of ICU stay, median days (range)	13 (4–15)	21 (…)	7 (2–12)
Mechanical ventilatory support			
No. (%) of subjects requiring mechanical ventilation	2 (16.7)	1 (16.7)	1 (10.0)
Duration of mechanical ventilatory support, median days (range)	4.5 (4–5)	17 (…)	4 (…)
No. (%) of patients who died	1 (8.3)	0 (0)	2 (20.0)
Diagnosis, no. (%) of patients			
Otitis media	3 (25.0)	0 (0)	0 (0)
Pharyngitis	0 (0)	0 (0)	0 (0)
Bronchiolitis/bronchitis	7 (58.3)	0 (0)	6 (60.0)
Pneumonitis	8 (66.7)	3 (50.0)	4 (40.0)
Common cold	0 (0)	2 (33.3)	0 (0.0)
Flulike syndrome	0 (0)	3 (50.0)	4 (40.0)
Symptom			
Feverishness/chills	11 (91.7)	5 (83.3)	8 (80.0)
Cough	9 (75.0)	5 (83.3)	10 (100)
Tachypnea/dyspnea	10 (83.3)	3 (50.0)	7 (70.0)
Wheezing/stridor	6 (50.0)	0 (0)	3 (30.0)
Rhinorrhea	4 (33.3)	2 (33.3)	3 (30.0)
Sore throat	1 (8.3)	3 (50.0)	1 (10.0)
Myalgia	0 (0)	1 (16.7)	1 (10.0)
Headache	0 (0)	1 (16.7)	0 (0)
Rash	2 (16.7)	0 (0)	0 (0)

NOTE. Data are no. (%) of subjects, unless otherwise indicated. Subjects were consecutive patients who were retrospectively identified by hMPV isolation in a regional virology laboratory in Quebec City, Canada. ICU, intensive care unit.

experienced more dyspnea and wheezing than did younger adults, and those with cardiopulmonary conditions were ill for nearly twice as long as younger adults. Among 10 retrospectively identified, infected patients aged >65 years who were hospitalized at our institution, 4 developed pneumonitis and 2 died (table 2) [7]. Of note, all 10 patients had ⩾1 underlying illness, such as leukemia/lymphoma and chronic cardiovascular, pulmonary, or neurologic diseases. Collectively, available data indicate that the clinical presentation of hMPV is very similar to that of hRSV, with more severe outcomes in infants, elderly subjects, and persons with underlying diseases [31].

The detection of hMPV in special settings merits further discussion. Preliminary data suggest that, similar to hRSV, hMPV infection may have a more fulminant course in severely immunocompromised individuals. Our group reported the death of a 17-month-old girl with acute lymphoblastic leukemia

due to severe pneumonitis and respiratory insufficiency [18]. Although no autopsy was performed, an NPA obtained before death revealed the presence of hMPV only. Of interest, this child had presented with a first episode of bronchiolitis caused by hMPV 1 year earlier, and the 2 viral isolates were of different lineages (groups), highlighting the possibility of rapid reinfections in immunocompromised hosts. Similarly, hMPV was the sole pathogen identified in the NPA obtained from a hematopoietic stem cell transplant recipient who died of progressive respiratory failure after an upper respiratory prodrome [32].

The presence of a copathogen may also lead to more-severe hMPV infections. A report from England identified hMPV in 21 (70%) of 30 bronchoalveolar fluid specimens obtained from infants with severe hRSV bronchiolitis requiring ventilatory support, raising the possibility that hMPV might be a determinant of hRSV disease severity [33]. Unfortunately, no data regarding dual infections in less severe hRSV disease were available for comparison. In contrast, the rate of bronchopneumonia was not altered by the presence of a second respiratory virus in Italian infants with hMPV infection [20].

Finally, the possible synergistic effect between hMPV and a new coronavirus has been recently postulated during outbreaks of severe acute respiratory syndrome (SARS) in Canada and Hong Kong [34, 35]. In a study of 48 patients with probable SARS in Hong Kong, Chan et al. [34] found evidence of hMPV infections by nested PCR in 25 cell cultures (52%) inoculated with NPA samples. In fact, hMPV was more frequently isolated than the SARS coronavirus in the latter study. This report is surprising, considering the results of experimental infections in macaques, in which severe multifocal pulmonary consolidation was induced by the SARS coronavirus only, with no exacerbation after subsequent infection with hMPV [36].

LABORATORY DIAGNOSIS

hMPV growth in cell culture is fastidious; this may be one reason for its late identification. Most studies have reported reliable cytopathic effects only in tertiary monkey kidney or LLC-MK2 cells [4, 6, 7]. The cytopathic effect is variable, with some strains inducing hRSV-like syncytia formation and others inducing focal rounding and cell destruction (figure 3). Typically, the cytopathic effect is displayed more than 10–14 days after inoculation (mean time was 17 days in our laboratory). Confirmation of hMPV cytopathic effect is achieved by RT-PCR testing of infected supernatants in the absence of commercially available antibodies. A recent report by Chan et al. [34] indicated that hMPV could replicate more efficiently in human laryngeal carcinoma (HEp-2) cells, despite the absence of cytopathic effect. In that instance, presence of hMPV was confirmed by direct testing of HEp-2 cell culture supernatants by RT-PCR or subsequent passage on LLC-MK2 cells to observe cytopathic effect.

Because of the unavailability of rapid antigen detection tests and because of the slow and restrictive viral growth, RT-PCR has become the method of choice for the diagnosis of acute hMPV infection. Most PCR protocols reported to date have relied on amplification of the L, N, or F gene (reviewed in [37]), with primer sequences mainly derived from the prototype strain 001 from The Netherlands (GenBank accession number AF371337). Because of the existence of 2 hMPV lineages with

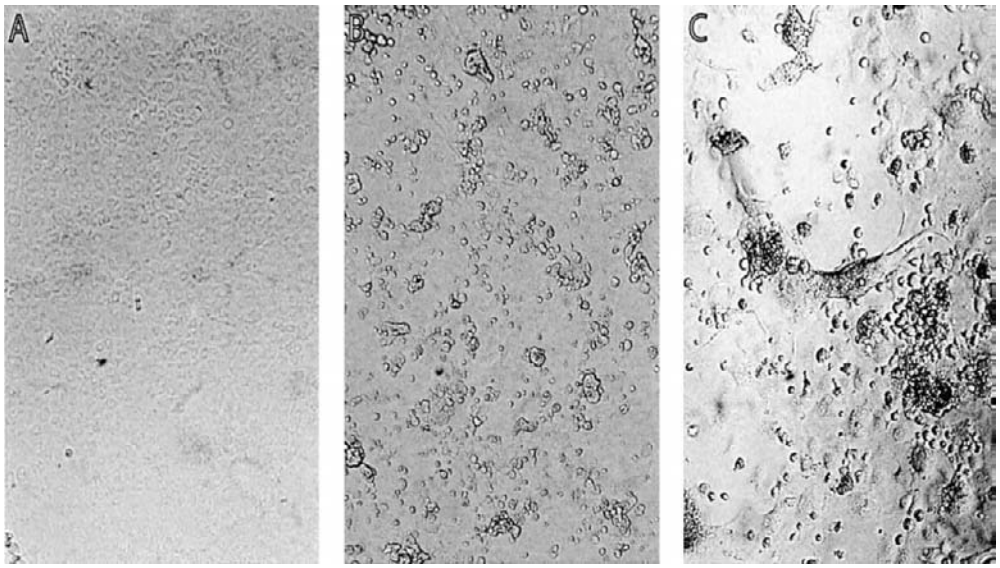

Figure 3. Microscopic studies of uninfected *(A)* and human metapneumovirus (hMPV)–infected LLC-MK2 cells. Note the various cytopathic effects induced by different hMPV strains consisting of small round cells *(B)* and large, respiratory syncytial virus–like syncytia *(C)*.

significant genetic variability within each group, hMPV detection may be underestimated when inadequate primers are selected for PCR amplification. We have recently reported that RT-PCR assays targeting the N and L genes, which code for 2 internal viral proteins, are best suitable for hMPV diagnosis [37]. Rapid and sensitive hMPV assays based on the real-time PCR methodology have recently been described, allowing amplification and detection of this pathogen in ≤2 h [37, 38].

Serological testing only permits a retrospective diagnosis. Because infection is almost universal in childhood, a seroconversion or a ≥4-fold increase in antibody titers must be demonstrated to confirm recent infection. The few serological surveys for hMPV were based on an indirect immunofluorescence assay using hMPV-infected cells [4, 16]. A home-brew ELISA method has also been developed using cell lysates of hMPV [21]. Clearly, simpler ELISA tests using viral proteins possibly derived from the 2 main groups are needed to conduct large serological surveys in many parts of the world.

PREVENTION AND TREATMENT

No vaccines, chemotherapeutic agents, or antibody preparations are currently approved for the prevention or treatment of hMPV infection. Recently, Wyde et al. [39] showed that ribavirin and a polyclonal intravenous immunoglobulin (IVIG) preparation had equivalent in vitro activity against hMPV and hRSV. In contrast, no activity against hMPV was conferred by palivizumab, a humanized monoclonal antibody directed against the F protein of hRSV. Despite the unavailability of animal studies and the toxicity related to ribavirin administration, the combined use of IVIG and ribavirin could be envisaged for treating severe hMPV infection in immunocompromised patients, as has been reported for hRSV infection [40, 41]. Furthermore, until an effective vaccine is developed, another interesting strategy could consist in the development of high-titered IVIG preparations with activity against hMPV.

CONCLUSIONS

The recent identification of a presumably old viral pathogen is an exciting development in the field of respiratory viruses. Available data indicate that hMPV appears to be a significant cause of both upper and lower respiratory tract infections in young children. hMPV reinfections seem to be frequent and could also lead to devastating complications in elderly subjects and immunocompromised hosts, although more studies with adequate control groups are needed to confirm this. Overall, the epidemiological and clinical features of hMPV infection appear to be similar to those of hRSV, although differences have been noted. It is important to mention that many fundamental questions related to viral pathogenesis and the host's specific immune response still remain to be answered.

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Note added in proof. Human metapneumovirus was recently isolated from previously virus-negative nasal wash specimens from 49 (20%) of 248 young children consulting at the Vanderbilt Clinic (Nashville, TN) for lower respiratory tract illnesses [44]. Extrapolation of these results to the entire cohort of patients observed at this institution over a 25-year period (1976–2001) suggests that the prevalence of human metapneumovirus infection was ~12% in all outpatient children with lower respiratory tract diseases.